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HORSERADISH MOSAIC [1]

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INTRODUCTION

In 1932, Dana and McWhorter (*2*)[3] published a brief account of a mosaic disease of horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.) in Oregon. They described symptoms of the disease and successfully transmitted the virus to turnip and mustard. In 1936, Kadow and Anderson [4] described "brittle root" of horseradish in Illinois, the effects of which resembled those produced by the beet curly top virus on this host. In a monograph of horseradish diseases in the United States, Kadow and Anderson (*4*) briefly described the mosaic disease. Horseradish mosaic has also been reported from Europe (*1*). In none of the above reports was any study made of the viruses involved. Furthermore, the certainty and extent of virus infection of horseradish stocks in this country has been a moot question.

The writer made a survey of horseradish plantings in various sections of the United States, and from mosaic-infected plants several apparently related viruses were isolated. The present paper reports investigations of the host range of these viruses, the symptoms they produce, and their physical properties.

MATERIALS AND METHODS

In table 1 is given the source of all the horseradish virus isolates that were studied in detail. Inoculations from naturally infected horseradish were made first to cabbage (*Brassica oleracea* var. capitata L., var. Jersey Queen), then to tobacco (*Nicotiana tabacum* L., var. Connecticut Havana No. 38), and finally to cabbage again. Passage through tobacco was used to separate strains of the turnip virus 1 group (*13*) from any strain of the cauliflower virus 1 group (*8*) in case horseradish mosaic should consist of such a complex as cabbage mosaic does (*13*). Extracts of the original inoculations to cabbage were heated for 10 minutes at 65° C. and reinoculated to cabbage to obtain viruses of the cauliflower virus 1 group free from those of the turnip virus 1 group. It might be stated here that in no instance has a strain of the former group ever been isolated from horseradish.

[1] Received for publication October 29, 1947.

[2] Appreciation is expressed to Eugene Herrling for making the photographs used in this paper.

[3] Italic numbers in parentheses refer to Literature Cited p. 114.

[4] KADOW, K. J., and ANDERSON, H. W. BRITTLE ROOT OF HORSERADISH IN ILLINOIS. U. S. Bur. Plant Indus., Plant Dis. Rptr. 20: 288. 1936. [Processed.]

For comparative purposes, cabbage virus A (*13*) was included in all and the cabbage black ring virus (*12*) in many of the parallel inoculations. Symptoms and classification of these strains have been detailed previously (*6*, *13*).

TABLE 1.—*Source, designation, and original symptoms produced by all horseradish isolates studied*

Locality from which originally obtained	Isolate designation	Original leaf symptons of diseased horseradish in original collection
Snohomish, Wash	HR1	Yellow-green mottling.
Eau Claire, Wis	HR2	Do.
Mt. Vernon, Wash	HR3	Yellow mottling and black ring necrosis.
St. Louis, Mo.[1]	HR4	Yellow mottling.
Burlington, Wash	HR5	Black ring necrosis.
Do	HR6	Yellow mottling.
Green Bay, Wis	HR7	Yellow-green mottling.

[1] Sent to the writer by Dr. C. M. Tucker.

All experiments were carried out in a greenhouse in which the air temperature was kept at 65° to 75° F. except as described under the section on temperature relations. Frequent fumigations were made to keep plants free of insects. In the host range studies four or five plants were inoculated with each virus in each test and an equal number were kept as controls. Many tests were repeated one or more times and those involving members of *Brassica oleracea* were repeated several times. The presence or absence of a virus in any host was confirmed by recovery inoculations to tobacco on which a characteristic symptom was produced. Inoculations were made by the rubbing method and carborundum was used regularly as an abrasive. Stock cultures of the virus isolates were carried in cabbage in aphid-proof cages. Property studies were made according to methods recently described (*13*).

SYMPTOMS AND HOST RANGE

SYMPTOMS ON HORSERADISH

In naturally infected horseradish plants, symptoms develop in the spring first as a chlorotic, blotchy mottling which may or may not be preceded by vein clearing (fig. 1, *A*). Leaves become generally chlorotic and much dwarfed. With age the mottling may become quite diffuse with chlorotic lesions losing their distinctiveness and becoming bright yellow instead of yellow green (fig. 2). Necrotic ringing is common in late season, the necrosis developing at the periphery of the chlorotic spots. Necrotic flecking and streaking of petioles (fig. 2) and leaf veins is very prevalent, especially in late season. Such necrosis extends through the roots, giving them a flecked appearance in transection.

A very marked development of filiform leaves often has been reported for horseradish and it has been suggested that this condition may be an effect of virus infection (*4*). Often a plant produces quite broad leaves and later becomes pinnatified; ultimately all its leaves may be reduced to extreme fern-leaf structures. The writer has found this condition to be very prevalent in commercial horse-

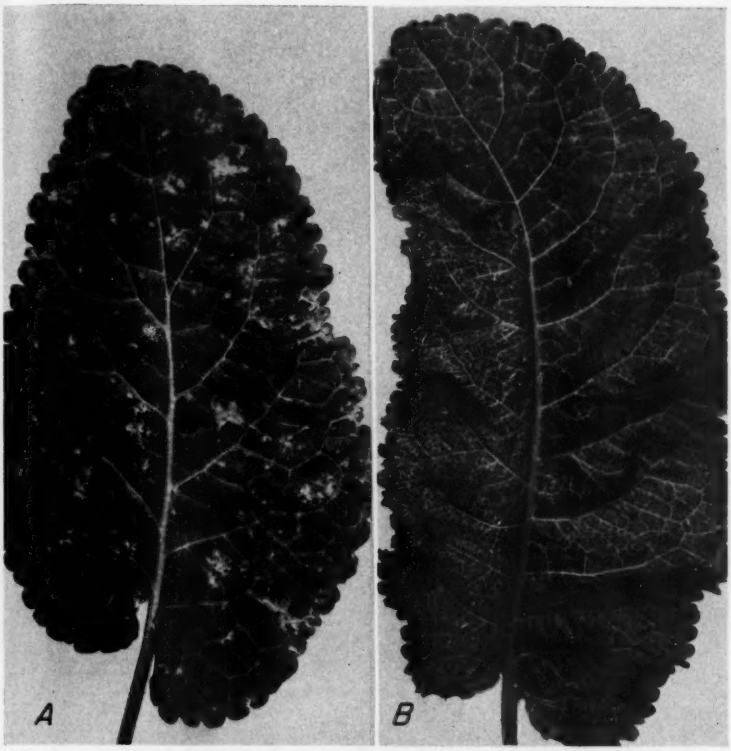

FIGURE 1.—Early systemic symptoms on horseradish naturally infected with mosaic. *A*, blotchy, chlorotic mottling; *B*, chlorotic vein clearing.

radish plantings but has also found it among plants from which no virus could be isolated (fig. 3), so its relationship to the mosaic disease, if any, is not clear.

SYMPTOMS ON OTHER HOSTS

The symptoms of the various strains are given in brief in table 2. Some of the cardinal points of similarity and contrast can be pointed out here. On members of *Brassica oleracea* the horseradish isolates had incubation periods 7 to 14 days longer than cabbage virus A and their rate of development after symptom manifestation was slower. On all members of *B. oleracea*, cabbage A and the black ring virus produced a coarse, diffuse, distorting mottle, while the horseradish isolates produced slowly developing chlorotic rings and ring spots with no diffuse mottling. Type symptoms as produced on broccoli are shown in figure 4.

On cabbage, the horseradish isolates produced symptoms more nearly approaching those of virus A than on cauliflower, broccoli, or brussels sprouts. On cauliflower the horseradish isolates produced

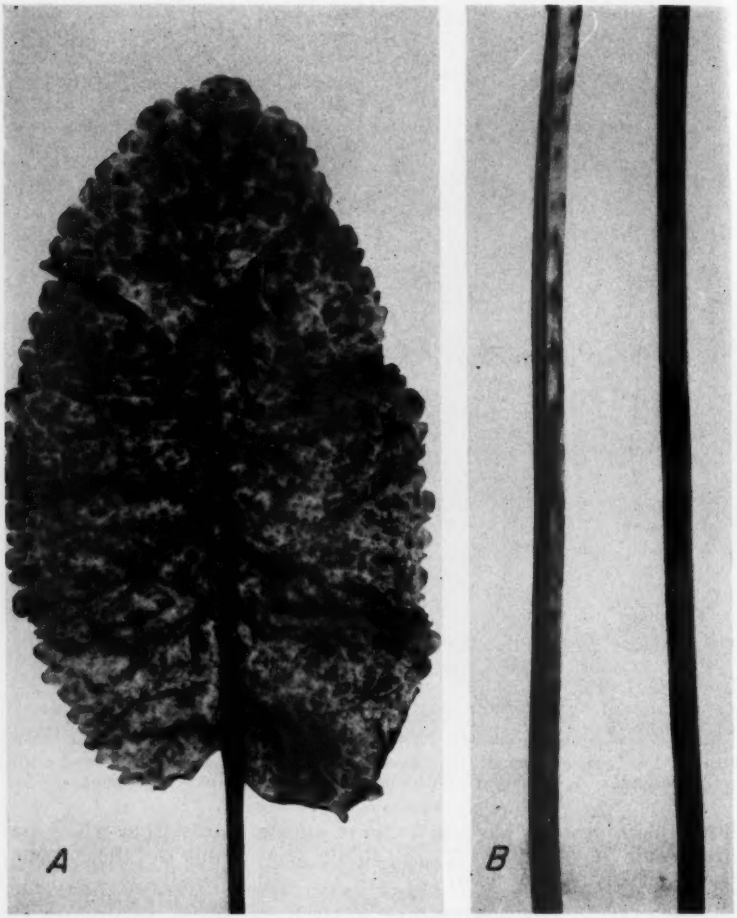

FIGURE 2.—Late season symptoms of horseradish naturally infected with mosaic. *A*, diffuse, yellow mottling; *B*, black streaking of petioles. Compare mottling with that shown in figure 1.

only chlorotic and necrotic rings. Although these isolates had a group reaction, some differences between them were noticeable. HR2 nearly always caused more pronounced symptoms which appeared more quickly than those of others in the group. It usually infected a high percentage of inoculated plants. HR1 did not infect cauliflower and only occasional plants of brussels sprouts. HR3 infected only occasional plants of cauliflower, while HR7 infected only occasional plants of both cauliflower and brussels sprouts.

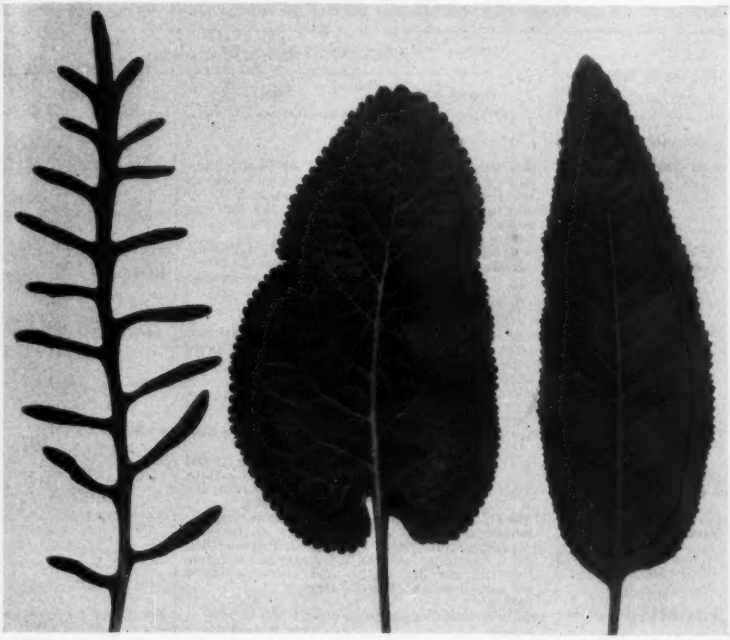

FIGURE 3.—Different leaf types obtained from a single horseradish plant of the Bohemian variety in which virus could not be demonstrated. Note the "fern-leaf" type which is often found in horseradish plantings.

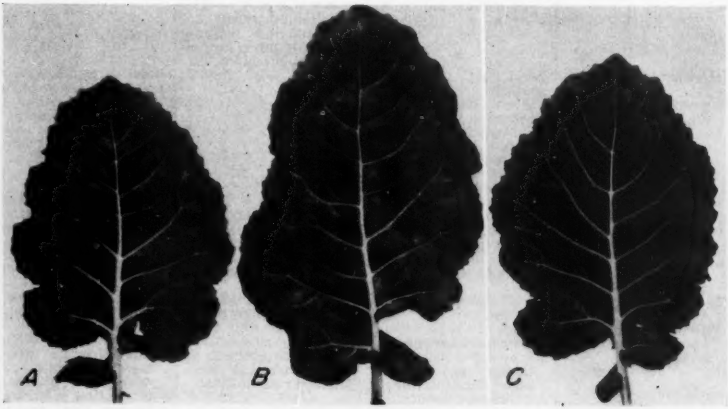

FIGURE 4.—Systemic symptoms on Italian Green Sprouting broccoli produced by *A*, cabbage virus A; *B*, cabbage black ring virus; *C*, horseradish isolate HR3. Note coarse, distorting mottling in *A* and *B* and chlorotic rings and ring spots in *C*. All horseradish isolates produced symptoms similar to those of HR3.

TABLE 2.—*Type symptoms produced on selected greenhouse hosts by cabbage virus A,*

Host family and species	Symptoms produced by isolate—		
	Cabbage A	HR1	HR2
AMARANTHACEAE			
Amaranthus caudatus L. (Love-lies-bleeding)	Local: Scattered chlorotic rings with tendency toward necrosis. Systemic: Sporadic necrotic rings and curvature of leaves.	Local: Pronounced necrotic rings; leaves rapidly abscise. Systemic: As for cabbage A.	As for HR1.
Celosia cristata L. (Cockscomb)	Local: None. Systemic: not observed.	Local: Pronounced necrotic lesions. Systemic: not observed.	As for HR1.
CHENOPODIACEAE			
Chenopodium album L. (Lambsquarters)	Local: Numerous pencil-point chlorotic lesions with only a tendency toward necrosis. Systemic: None.	Local: Numerous necrotic lesions; leaves rapidly abscise. Systemic: None.	As for HR1.
CRUCIFERAE			
Barbarea vulgaris R. Br (Upland cress)	None.	Local: Scattered chlorotic lesions. Systemic: Pronounced yellow-green mottling; moderate stunting and leaf distortion.	As for HR1.
B. juncea (L.) Coss (Indian mustard)	Local: Occasional necrotic lesions. Systemic: severe yellow-green mottling; severe stunting and leaf distortion; necrosis with age.	Local: As for cabbage A. Systemic: As for cabbage A but mottling yellower and stunting and necrosis more severe.	As for HR1.
B. oleracea var. *botrytis* L. (Broccoli, var. Italian green sprouting)	Local: Occasional chlorotic lesions. Systemic: Coarse, yellow-green mottling with considerable areas of diffuse chlorosis; sporadic necrosis on older leaves; marked stunting and distortion of young leaves.	Local: None. Systemic: Slowly developing chlorotic rings and ringspots never numerous enough to give mottling effect; some necrotic ringing on lower leaves; little stunting and no distortion; markedly different from cabbage A; low percentage of plants infected.	Local: As for cabbage A. Systemic: As for HR1, but chlorotic rings more numerous and develop more rapidly.
B. oleracea var. *botrytis* L. (Cauliflower, var. Early Snow ball)	Local: None. Systemic: Coarse yellow-green mottling with some tendency toward chlorotic ringing; some diffuse chlorosis; necrotic ringing on lower leaves with age; moderate stunting.	None.	Local: None. Systemic: Slowly developing small chlorotic rings, becoming necrotic; ultimately only necrotic ringing; mottling; slight stunting.
B. oleracea var. *capitata* L. (Cabbage, var. Jersey Queen)	Local: None. Systemic: Coarse diffuse, yellow-green mottling; slight irregular necrotic spots; moderate stunting and leaf distortion.	Local: None. Systemic: Slowly developing chlorotic rings; no coarse mottling; no necrosis; no distortion; much less severe than cabbage A.	As for HR1, except chlorotic rings more numerous and develop more quickly.
B. oleracea var. *gemmifera* DC. (Brussels sprouts, var. Long Island Improved)	Local: Sporadic chlorotic lesions. Systemic: Coarse yellow-green mottling with tendency for lesions to be regular and circular; some necrotic ringing; moderate stunting.	Local: As for cabbage A. Systemic: Slowly developing chlorotic rings and ringspots; no mottling; no necrosis; no stunting; only occasional plant infected.	Local: Scattered chlorotic lesions becoming necrotic. Systemic: As for HR1, but chlorotic rings more numerous and develop more quickly; no mottling as for cabbage A; high percentage of plants infected.
Capsella bursa-pastoris (L.) Medic (Shepherds-purse)	Local: Yellow chlorotic lesions and streaking of veins. Systemic: Yellow mottling; severe stunting and rosetting with curvature of leaves.	As for cabbage A, but slightly more severe.	As for HR1, but more severe; lethal.

together with variations from "type symptoms" produced by the horseradish isolates

Symptoms produced by isolate—Continued				
HR3	HR4	HR5	HR6	HR7
As for HR1	As for HR1	As for HR1	As for HR1	As for HR1
Local: Pronounced chlorotic lesions. Systemic: Not observed.	Local: Scattered necrotic lesions. Systemic: Not observed.	As for HR1	As for HR3	As for HR1
As for HR1	As for HR1	As for HR1	As for HR1	As for HR1
As for HR1, but more severe.	As for HR3	As for HR3	As for HR3	Not tested.
Local: None Systemic: As for HR1.	As for cabbage A	As for HR1	As for HR1	As for HR1
As for HR1, but chlorotic ringspots more conspicuous.	As for HR3	As for HR3	As for HR3	As for HR1
Local: None Systemic: Plants rarely infected; symptoms as for HR2.	As for HR2	As for HR2	As for HR2	As for HR3
As for HR2	At first as for HR2, but chlorotic rings numerous enough to produce mottling effect; intermediate between cabbage A and HR2.	As for HR4	As for HR4	As for HR4
As for HR2	As for HR2	As for HR2	As for HR2	As for HR1
As for HR2, but plants killed more quickly.	As for HR2	As for HR2	As for HR2	Not tested.

TABLE 2.—*Type symptoms produced on selected greenhouse hosts by cabbage virus A,*

Host family and species	Symptoms produced by isolate—		
	Cabbage A	HR1	HR2
CRUCIFERAE—continued			
Cheiranthus allionii Bailey (Siberian wallflower).	Local: None. Systemic: Very mild mottling; no necrosis; very little stunting; no distortion.	As for cabbage A except more severe mottling, stunting, and distortion; symptoms appear several days earlier than for cabbage A.	Local: None. Systemic: Marked vein clearing and mottling; severe stunting; severe puckering of leaf laminae and inrolling of growing tips; more severe than HR1.
C. cheiri L. (Wallflower)	Local: None. Systemic: Mild mottling, stunting, and curvature of growing tip.	As for cabbage A except symptoms appear earlier.	As for cabbage A but much more severe.
Lepidium virginicum L. (Wild peppergrass)	Local: Scattered brown necrotic lesions. Systemic: Golden-yellow bronzing of leaves; moderate stunting; rosetting.	As for cabbage A.	Local: As for cabbage A. Systemic: As for cabbage A but much more severe; lethal.
Lobularia maritima Desv. (Sweet alyssum)	Local: None. Systemic: Slight mottling; very slight stunting and leaf distortion.	Local: None. Systemic: Slight mottling, stunting, and distortion; slight necrosis; more severe than cabbage A.	Local: None. Systemic: Severe mottling, stunting. leaf distortion, and necrosis; much more severe than HR1.
Malcomia maritima Ait. (Virginian stock)	Local: None. Systemic: Slight yellow mottling; no necrosis; slight stunting and puckering of leaves.	Local: None. Systemic: Slight yellow mottling; necrotic streaking of veins; necrotic spots between veins; leaves not puckered but growing tip distorted; much more severe than cabbage A.	As for HR1.
Matthiola incana var. *annua* (L.) Voss (annual stock, var. dwarf flowering 10 weeks)	Local: Scattered necrotic lesions. Systemic: Vein clearing and mottling; slight etched pattern of necrosis; moderate stunting.	Local: Numerous necrotic lesions. Systemic: Vein clearing and mottling; severe bleaching of leaves becoming necrotic; severe stunting; quicker developing symptoms and much more severe than cabbage A.	As for HR1.
Sisymbrium officinale (L.) Scop. (Hedgemustard)	Local: None. Systemic: Slight mottling; slight necrosis; moderate stunting and inrolling of young leaves.	As for cabbage A but slower in developing.	Local: Numerous necrotic lesions. Systemic: Mottling as for cabbage A but much more severe necrosis; lethal.
Thlaspi arvense L. (Pennycress)	Local: General chlorosis, becoming necrotic. Systemic: Pronounced vein clearing, mottling, stunting, and distortion of young leaves.	Local: Pronounced necrotic lesions. Systemic: As for cabbage A but necrotic; lethal.	As for HR1 but necrosis more severe.
SOLANACEAE			
Solanum integrifolium Poir. (Chinese eggplant)	Local: Necrotic leaves and necrotic streaking of veins. Systemic: Necrotic leaves and necrotic streaking of veins.	None.	As for cabbage A.
S. triflorum Nutt.	Local: None. Systemic: Coarse yellow-green mottling; slight parchmentlike necrosis; severe stunting and distortion of young leaves.	Local: None. Systemic: Broad chlorotic spots giving slight mottling; necrosis as for cabbage A; slight stunting and distortion; much less severe than cabbage A.	As for cabbage A but much more severe necrosis and distortion.

together with variations from "type symptoms" produced by the horseradish isolates—Con

Symptoms produced by isolate—Continued				
HR3	HR4	HR5	HR6	HR7
As for HR2	As for HR2	As for HR2	As for HR1	As for HR 2
As for HR1	As for HR2	As for HR1	As for HR2	As for HR1.
As for cabbage A but numerous local lesions.	Local: As for cabbage A. Systemic: Very little bronzing of leaves, stunting, or distortion; practically symptomless and markedly less severe than others.	Not tested	Not tested	Not tested.
As for HR2	As for cabbage A	As for cabbage A.	Not tested	As for HR2.
As for HR1	As for HR1 but much more severe.	Not tested	As for HR4	Not tested.
As for HR1	As for HR1	As for HR1	As for HR1	As for HR1.
As for HR2	As for HR1 but slightly more severe.	As for HR1 but slightly more severe.	Not tested	As for HR1.
As for HR2	As for HR1	As for HR2	Not tested	Not tested.
None	None	None	None	None.
As for cabbage A; less severe than HR2 but more severe than HR1.	As for HR3	As for HR1	As for cabbage A.	Not tested.

On *Amaranthus caudatus*, *Chenopodium album*, and pennycress, cabbage virus A produced chlorotic primary lesions with occasional necrosis, while all horseradish isolates developed conspicuous necrotic local lesions. On annual stock all horseradish isolates had incubation periods 3 to 5 days shorter than cabbage A and the black ring virus and uniformly caused more severe symptoms than either of the cabbage viruses (fig. 5).

On *Solanum integrifolium* only cabbage A and HR2 produced symptoms.

Although the reactions of the horseradish isolates were quite similar as a group, there were distinct differences. Thus HR1 was not infec-

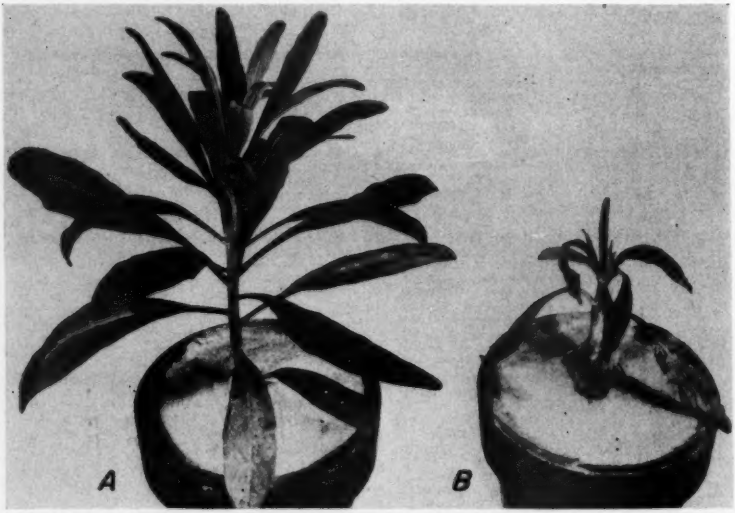

FIGURE 5.—Systemic symptoms on annual stock produced by cabbage black ring virus (*A*) and horseradish isolate HR1 (*B*). Note much more severe stunting caused by HR1. All horseradish isolates were markedly more virulent than cabbage A and cabbage black ring viruses.

tious to cauliflower; HR2 alone infected *Solanum integrifolium*; HR4 was much less severe on pennycress than others. Other minor differences serving to differentiate between the horseradish isolates can be found in table 2 and in the section on temperature relations.

HOST RANGE

The symptoms on selected greenhouse plants in response to each virus are given in table 2. In making the host range study species to be tested were chosen which, on the basis of previous experience, might best differentiate between the virus isolates. Consequently, no broad host range including several families was tested. It is to be noted that all strains were pathogenic to all cruciferous species tested except *Barbarea vulgaris* and cauliflower. Cabbage virus A did not infect *B. vulgaris* and isolate HR1 did not infect Snowball cauliflower. A European variety of cauliflower (Dwarf Erfurt), however, was

infected by HR1, but several attempts to infect the lot of Snowball cauliflower used were unsuccessful. Among noncruciferous plants tested all strains had identical host ranges except *Solanum integrifolium*, which was infected by cabbage virus A and horseradish isolate HR2 but not by the others. Thus only minor differences occurred in the host ranges.

EFFECT OF TEMPERATURE ON EXPRESSION OF SYMPTOMS

Pound and Walker (*6*) showed that symptom expression and virus activity of cabbage virus A and the black ring virus were markedly affected by air temperatures to which infected plants were exposed. As a means of further differentiating between the cabbage and horseradish viruses and between individuals of the latter group, symptom development was studied on selected hosts at different air temperaures.

Horseradish

Naturally infected horseradish roots from the same source were planted in greenhouse compost and forced at air temperatures of 28°, 24°, 20°, and 16° C. Symptoms appeared first at 28° and last at 16° C. At all temperatures the first sign of the disease was a blotchy mottling which was soon followed by pronounced vein clearing and vein banding. Plants became very chlorotic. Within 3 weeks symptoms on plants at 28° and 24° became less pronounced and ultimately were almost completely masked at 28°. The severe chlorosis was replaced by normal dark-green growth and ultimate growth was much greater than at lower temperatures. No necrotic ringing and only occasional black streaking occurred. At 20° and 16° the vein clearing and mottling were persistent, but the blotchy mottling was replaced by a fine diffuse mottling and chlorotic lesions became conspicuously bright yellow. Necrotic ringing and black streaking were very prevalent.

Broccoli and Cabbage

All horseradish isolates, cabbage A, and cabbage black ring viruses were tested on plants incubated at 28° and 16° C. At 28° all viruses produced symptoms in 7 to 10 days as vein clearing followed by mottling. The mottling of cabbage A and the black ring viruses rapidly became severe, with considerable diffuse chlorosis and leaf distortion. Chlorotic lesions were irregular in size and shape. All horseradish isolates produced chlorotic rings and ring spots with little diffuse chlorosis or leaf distortion.

At 16° C. virus A produced a systemic diffuse mottling much less severe than at 28° but one in which chlorotic lesions were irregular and not always individually distinct. Mottling of the black ring virus was similar but lesions were more circular and distinct; some necrosis occurred. All horseradish isolates produced scattered chlorotic flecks or chlorotic rings with less ring spotting than at 28°. The small flecks were characteristic of HR1; the others produced chlorotic rings. The rings were most numerous for HR2 but not numerous enough to merge. With HR7 the rings were yellow in color and tended to be necrotic. HR3 infected only occasional plants. Thus all horseradish isolates were easily distinguishable from the cabbage viruses at this temperature (fig. 6).

FIGURE 6.—Systemic symptoms produced at 16° C. on Italian Green Sprouting broccoli by *A*, cabbage virus A; *B*, horseradish isolate HR1; *C*, horseradish isolate HR2; *D*, horseradish isolate HR7. Note diffuse mottling with cabbage virus A, chlorotic flecks with HR1, numerous chlorotic rings with HR2, and large yellow rings with HR7.

CAULIFLOWER

All viruses were tested on Early Snowball cauliflower incubated at 28° and 16° C. At 28° the cabbage A and cabbage black ring viruses produced a coarse mottling, beginning in 7 days. Both ultimately produced diffuse chlorosis with chlorotic lesions irregular in size and shape. Isolate HR2 developed symptoms in 2 weeks as systemic chlorotic rings numerous enough to give a mottling effect, but no diffuse chlorosis. Small blisterlike pockets on the leaf surface were unique with this isolate. Isolates HR3, HR4, HR5, HR6, and HR7 produced scattered chlorotic rings on occasional plants and often no more than one lesion per leaf. HR1 was not infectious and could not be recovered from the symptomless plants.

At 16° the cabbage A and black ring viruses produced a coarse mottling in 14 days. Chlorotic lesions tended to be small and circular as compared to those produced at 28°. Necrosis later developed at the periphery of the lesions to produce a conspicuous black-ringing pattern. Lesions of the black ring virus were more circular than those of virus A and stunting and necrosis were more severe. Isolate HR2 produced symptoms in 21 days as scattered, small, chlorotic rings which rapidly became necrotic, producing in effect only systemic necrosis. HR3 produced only target-board necrotic lesions on inoculated leaves. Isolates HR4, HR5, HR6, and HR7 produced only small, systemic, necrotic rings which were similar to those produced by HR2 but which were usually not preceded by chlorotic rings. No infection occurred with HR1. Representative symptoms of the various isolates at 16° are shown in figure 7.

Thus all horseradish isolates were markedly different from the two cabbage viruses at both temperatures.

NICOTIANA GLUTINOSA

Observations were made on all horseradish isolates and cabbage virus A at 28°, 24°, 20°, and 16° C. At 28° all but HR3 and HR7 produced brown necrotic local lesions around which developed concentric etched-like rings. Systemic symptoms of cabbage A, HR1,

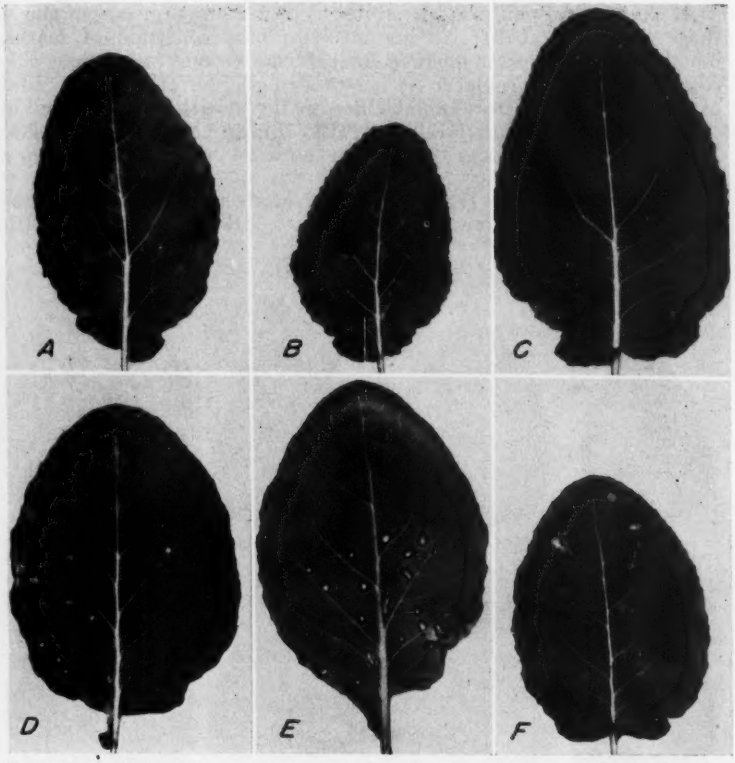

FIGURE 7.—Systemic symptoms 4 weeks after inoculation on Early Snowball cauliflower at 16° C. produced by *A*, cabbage virus A; *B*, cabbage black ring virus; *C*, horseradish isolate HR1; *D*, horseradish isolate HR2; *E*, horseradish isolate HR4; *F*, horseradish isolate HR7. Note chlorotic mottling for the cabbage viruses and necrotic ringing for the horseradish isolates except HR1, which caused no infection.

HR2, and HR5 consisted of zonate etched-like rings which developed very slowly and never occurred on the youngest leaves. These were numerous for cabbage A, moderate in number for HR2, and very few for HR1 and HR5. Isolates HR3, HR4, HR6, and HR7 produced no systemic symptoms.

At 24° cabbage A, HR1, and HR2 produced broad cholorotic primary lesions which soon become necrotic. HR3, HR5, and HR6 produced similar lesions except for the necrosis, while HR4 and HR7 produced no primary symptoms. Systemic symptoms consisted of circular chlorotic lesions with a slight tendency toward zonation. These rapidly became very necrotic with HR2 but only partially necrotic with cabbage A. Isolates HR5, HR6, and HR7 either produced no systemic symptoms or only occasional chlorotic spots while HR1, HR3, and HR4 remained entirely symptomless.

At 20° and 16° all isolates produced primary symptoms similar to those produced at 24°. Systemic symptoms consisted of marked mottling and moderate necrosis for cabbage virus A; numerous chlorotic flecks rapidly replaced by necrotic ringing for HR2 and to a less extent for HR6; scattered small chlorotic flecks with slight necrosis for the other isolates, with HR1 and HR7 being almost symptomless. Representative symptom types produced at 24° and 20° are shown in figure 8.

Thus on this host, HR2 of the horseradish isolates was nearest cabbage virus A but yet was distinct. HR1 and HR7 were the

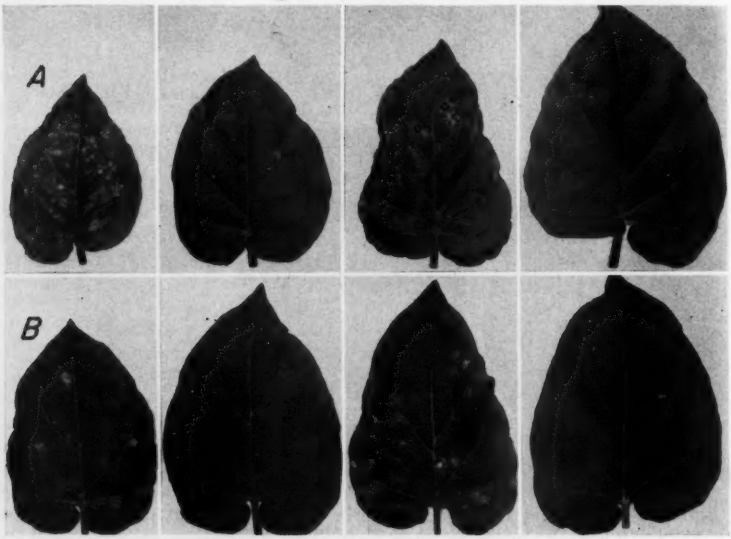

FIGURE 8.—Systemic symptoms produced on *Nicotiana glutinosa* at 24° C. (*A*) and 20° C. (*B*) by virus isolates, left to right; cabbage A, HR1, HR2, and HR3. Note that HR2 was much more severe than other horseradish isolates.

least severe and were identical with each other. Isolates HR3, HR4, HR5, and HR6 were intermediate between cabbage virus A and HR2 and were not easily distinguishable from each other.

PROPERTIES OF THE VIRUSES

Property studies were run only on horseradish isolates HR1 and HR2. It was thought that these isolates would adequately represent the group in view of relationships already shown in symptoms and host range, and in immunological reactions given in the following section. The results of the property studies are presented in table 3. It can be seen that properties of the isolates tested were identical with those given for cabbage virus A (*13*) except for slightly less tolerance to aging in vitro.

TABLE 3.—*Physical properties of virus isolates HR1 and HR2 as determined by the number of local lesions formed on inoculated tobacco*

[10 half leaves inoculated in each trial with each isolate]

Type and degree of treatment	Lesions produced by isolate [1]—	
	HR1	HR2
Dilution:	*Number*	*Number*
0	847	599
1–10	412	554
1–100	132	265
1–1,000	11	35
1–2,000	7	7
1–4,000	3	1
1–6,000	1	1
1–8,000	2	1
1–10,000	2	0
1–15,000	0	0
Heating for 10 minutes (°C.):		
None	814	578
52	293	257
54	106	180
56	31	15
58	15	6
60	0	1
62	0	0
Aging in vitro at 20 ° C.:		
None	847	599
1 day	406	437
2 days	1	46
3 days	0	3
4 days	0	0
5 days	0	0
6 days	0	0
7 days	0	0
8 days	0	0

[1] Values are averages of 2 trials each; with each isolate trials 1 and 2 were inoculated in half-leaf comparisons.

HOST IMMUNITY REACTIONS

In table 2 it was shown that the cabbage virus A and HR2 both gave necrotic lesions on inoculated leaves of *Solanum integrifolium* while all other isolates were noninfectious to this host. The following immunological tests were set up with this host as an assay plant. Groups of 10 plants of Dwarf Essex rape were inoculated with one or another of the following horseradish isolates: HR1, HR3, HR4, HR5, HR6, and HR7. Upon the development of systemic symptoms all plants were reinoculated with cabbage virus A, only leaves showing systemic symptoms being inoculated. At the same time an equal number of healthy plants of the same age similarly were inoculated with cabbage virus A. After systemic symptoms developed in these control plants, inoculum was taken from each group of plants and applied to healthy plants of *S. integrifolium*. Abundant local lesion development occurred on plants of *S. integrifolium* inoculated with sap taken from plants infected with cabbage virus A alone, but none occurred in any case where inoculum was taken from plants infected with a horseradish isolate and reinoculated with cabbage virus A (fig. 9).

In an identical test, isolate HR6 protected rape plants against infection from isolate HR2. As a further test inoculum was taken from the rape plants infected with the horseradish isolates and reinocu-

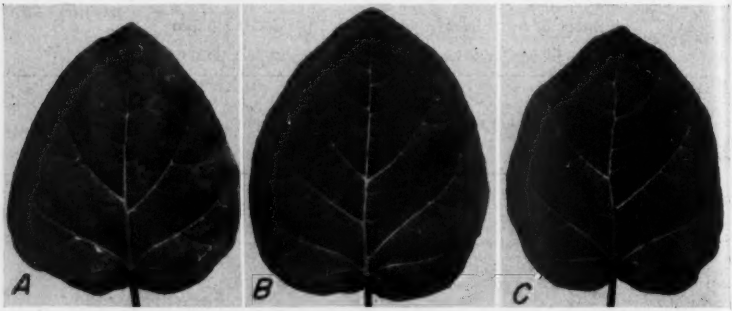

FIGURE 9.—Symptoms produced on inoculated leaves of *Solanum integrifolium* with extracts taken from rape plants used in the cross immunity tests of cabbage virus A and the horseradish isolates. Leaves from plants inoculated with: *A*, extract taken from rape infected with cabbage virus A alone; *B*, extract from rape infected with isolate HR1 alone; *C*, extract from rape infected with isolate HR1 and reinoculated with cabbage virus A.

lated with cabbage virus A or HR2 and transferred to Early Snowball cauliflower. Symptoms in each case were characteristic of the horseradish isolate originally applied, and in no instance were symptoms produced typical of cabbage virus A. As a still further test, inoculum was taken from the cauliflower plants involved and transferred again to *Solanum integrifolium*. No symptoms were produced except where cabbage virus A or HR2 alone was involved. These tests indicate a strain relationship between cabbage virus A and the horseradish isolates. The fact that HR6 protected against both cabbage virus A and HR2 indicates a strain relationship between the last two. Since cabbage virus A has been shown to be a strain of turnip virus 1 (*6*) these data are interpreted to mean that the horseradish viruses are also strains of turnip virus 1.

DISCUSSION

Commercial plantings of horseradish in Wisconsin, Illinois, Missouri, and Washington which were visited or sampled were found to be practically 100 percent mosaic-infected. Wild plantings in these areas similarly were infected although occasional virus-free plants were found. Since horseradish is propagated vegetatively this condition is not surprising. Growers in the East St. Louis and Chicago areas in past years have suffered heavy losses from "deterioration" trouble (*4*). Some have thought the trouble was due to virus infection and have endeavored to remedy the situation by growing plants for planting stock in isolated areas. Thus some 200 to 300 acres of horseradish are grown annually in Wisconsin to provide planting roots for the St. Louis and Chicago areas. The Wisconsin plantings are 100 percent infected with mosaic and the increased yields from stocks of such isolated plantings as reported by Kadow and Anderson (*4*) can hardly be attributed to absence of infection with such viruses as those described in this paper.

The identification of the horseradish isolates as strains of turnip virus 1 (*3*) is further manifestation of the variability of that virus.

The horseradish isolates are very similar to the cabbage strains of that virus and differ from them more in symptom intensity than in host range. Their physical properties are identical. It is notable that of the seven isolates selected for detailed study each was sufficiently different from the others and from other described strains to constitute a distinct strain. An extensive survey in horseradish would no doubt yield more strains.

Although the horseradish isolates differed from one another they had several group reactions that distinguished them from the cabbage viruses. Thus, on annual stock they were all markedly more severe; on members of *Brassica oleracea* they produced circular chlorotic or necrotic rings and ring spots instead of a diffuse mottling. It might be well to point out here how the horseradish viruses differ from other related viruses described on cruciferous plants other than the two cabbage viruses mentioned. Thus they differ from Tompkins' (*9*) turnip mosaic virus by their infectivity on brussels sprouts, broccoli, rape, and wild mustards. They differ from the radish mosaic virus (*11*) in their infectivity to rape, annual stock, dames violet, and their failure to produce systemic infection in tobacco. They differ from the two mosaic viruses of annual stock (*10*) and from turnip viruses T1, T6, and T9 (*5*) in their infectivity to cabbage, cauliflower, and brussels sprouts.

Similarly, the horseradish viruses can be separated from one another. HR1 was distinct from all others in its failure to infect cauliflower but HR3 was closely related to it in this regard. HR2 was quite distinct from the others in its infectivity to *Solanum integrifolium* and its increased severity on most hosts. HR4 was distinct from all others in its failure to produce pronounced symptoms on *Lepidium virginicum*. Other differences can be found by studying the host range table, pp. 102.

In no instance was a virus ever isolated from horseradish that resembled cauliflower virus 1 (*8*). Evidently strains of this virus are not infectious to horseradish although no inoculations to check this point were made. Conspicuous vein clearing and vein banding are characteristic symptoms of strains of the cauliflower virus 1 group and are also prominent symptoms of horseradish mosaic but are apparently produced in this case by the turnip virus 1 isolates.

Pound and Walker (*6*) have shown that cabbage virus A and the cabbage black ring virus produce more pronounced symptoms in cabbage and other hosts in general at 28° than at 16° C., and that, in cabbage, masking tends to occur at unfavorable temperatures. Furthermore they found the virus concentration in cabbage growing at 28° C. to be markedly greater than in plants growing at 16° C. (*7*), and they suggested that members of the turnip virus 1 group in general were favored by relatively high air temperatures. In this investigation infected horseradish roots (from a source not represented by any isolate reported herein in detail) quickly masked symptoms when forced at 28°, but maintained persistent vein clearing, mottling, and necrosis when forced at 16° C. A preliminary test indicated that the virus was in considerably higher concentration at 16° than at 28° C. This is the exact opposite of results obtained with cabbage virus A and cabbage black ring virus in cabbage. Whether this would be true of the horseradish viruses in general and in other hosts remains for further investigation. It is further indication, however, that the effect of temperature on virus disease development probably depends upon the

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specific host-virus complex and may be due to the temperature reaction of the host, the virus, or both.

SUMMARY

In this investigation seven virus isolates from horseradish collected from Wisconsin, Missouri, and Washington were studied in comparison with each other and with similar viruses occurring in cabbage. Horseradish plantings in these areas were found to be practically 100 percent virus-infected.

In properties and host range the horseradish viruses agreed very closely with cabbage virus A and the cabbage black ring virus, although slight differences occurred. Among the horseradish viruses, isolate HR1 did not infect Snowball cauliflower; HR2 alone infected *Solanum integrifolium*. When symptom type and intensity were considered the horseradish isolates differed markedly from each other and from the cabbage viruses.

By cross immunity tests the horseradish isolates were shown to have a strain relationship with cabbage virus A and are thus designated as strains of turnip virus 1.

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ANTIBIOTIC SUBSTANCES IN ONION IN RELATION TO DISEASE RESISTANCE[1]

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INTRODUCTION

Walker, Link, and associates (*1, 6, 7, 8, 13, 20*)[2] have shown that resistance of colored onions (*Allium cepa* L.) to smudge (*Colletotrichum circinans* (Berk.) Vogl.) and neck rots (*Botrytis allii* Munn and *B. byssoidea* J. C. Walker) is due primarily to toxic, water-soluble, phenolic substances in the dry outer scales associated with the plant pigments. Two of the materials have been identified as protocatechuic acid and catechol. More recently Jones, Walker, Little, and Larson (*5*) have shown that the degree of resistance, as measured by the smudge index, varies among varieties and progenies having the same gene for yellow color and between heterozygotes from crosses of a colorless variety with each of two colored varieties containing the same gene for color. It was suggested that secondary characters influence resistance of a given progeny to smudge and that possibly the antibiotic constituents of fleshy scales were concerned.

Walker (*12*), in 1918, reported on the inhibitive effect of volatile substances present in onion tissue upon germination of spores of *C. circinans*. In a more comprehensive study in 1923, he (*14*) reported that the volatile substances and the liquid from succulent onion tissue were toxic to spores. These studies were continued and the results published by Walker, Lindegren, and Bachmann (*18*). The fungicidal effect of the volatile substances, as well as of the substances remaining in the liquid, was tested on *C. circinans*, *B. allii*, and black mold organism *Aspergillus niger* Van Tiegh., and certain other organisms. They found that all the fungi tested were susceptible to the vapor given off by crushed onion tissue. They suggested that as the parasite invaded the host the toxic material became somewhat attenuated but that the invasion of the organism was retarded in proportion to its sensitivity. This was given as a possible explanation of the fact that the dry scales of white onions and the uncolored portions of dry scales of colored onions were readily invaded by *C. circinans* but the invasion of both white and colored succulent scales was distinctly slower than that by *B. allii* (*18*).

Other investigators have studied the bactericidal and bacteriostatic action of substances present in the tissue of onion and related plants.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 135.

Walton, Herbold, and Lindegren (*21*), found that the vapor from garlic (*Allium sativum* L.) was bactericidal to *Bacillus subtilis* Cohn, but that boiling and autoclaving rendered the vapor nontoxic. Lovell (*9*), 1937, found that 2 percent glycerin jelly, after being exposed to the vapor from crushed onion tissue, would not support growth by *B. subtilis*. He found also that emission of vapor increased with temperature from 10° to 37° C. Ingersoll et al. (*4*) found acrolein from garlic highly bactericidal and suggested that crotonaldehyde might be the toxic agent in onion. Fuller and Higgins (*3*) reported that onion extract vapor was toxic at certain concentrations to *Escherichia coli* (Migula) Castellani and Chalmers; *Staphylococcus aureus* Rosenbach, and *Bacillus subtilis*.

These previous studies suggested that the volatile substances present in the succulent scales of onion might contribute to their resistance to the smudge, neck rot, and black mold organisms. Especially does this thought asume significance when it is known that varieties of onions of the same color possess different degrees of pungency (*10*, *11*) and different incidences of smudge and neck rot. The present studies were designed to investigate further the relation of antibiotic substances in onion scales to disease resistance.

MATERIALS AND METHODS

Seeds of the onion varieties were obtained from H. A. Jones of the United States Department of Agriculture, Beltsville, Md. They were sown in flats in the greenhouse in late February and the seedlings were transplanted to the field in randomized blocks in mid-April. During the growing seasons, on dates indicated in table 1, the onions were inoculated by applying water suspensions of spores of *C. circinans*, *B. allii*, and *A. niger* to the exposed part of the bulbs and to the soil adjacent to the bulbs. The inoculum was applied in the evening and the plot was immediately irrigated by means of overhead sprinklers.

TABLE 1.—*Dates of inoculation of onion plots with spore suspensions of Colletotrichum circinans, Botrytis allii, and Aspergillus niger*

Organism	Dates of inoculation in—			
	1944	1945	1946	1947
C. circinans	July 1, 26	June 15, July 10, 28	June 27, July 10, 22	July 2, 24.
B. allii	Aug. 5, 19	July 30, Aug. 12, 28	July 18, 22, Aug. 1	July 2, 23.
A. niger		Aug. 28	Aug. 1	Aug. 22.

The onions were harvested in late August and early September, placed in mesh bags in shallow layers on slatted racks, and stored in an open shed. Diseases were noted in late October and the onions were then placed in a cold storage room maintained at 5° C. until further experiments were made. The smudge incidence was recorded by placing the bulbs in one of four arbitrary classes designated as follows: (1) those free from symptoms and signs; (2) those slightly diseased; (3) those moderately diseased; and (4) those severely diseased. These classes were assigned the numbers 0, 1, 2, and 3, respectively. An index for each lot was calculated by multiplying the number in each class

by the class number and dividing the total by the product of the highest class and the total number of bulbs in the lot; this figure was then multiplied by 100 to secure the index. The incidence of neck rot and black mold was expressed in percentage of bulbs affected and in one case a black mold index was calculated in the same manner as for smudge. Scale character was judged by placing the bulbs into the following three classes:

(1) *Good*—good protection by outer scales adhering well and remaining whole.
(2) *Poor*—poor protection of fleshy scales either through one, or at most two, thin, easily sloughing outer scales, or through splits in the outer scale exposing the fleshy scale.
(3) *Very poor*—the outer scales nearly or completely sloughed with the fleshy scale well exposed.

Onion juice was obtained by grinding the fleshy scales of 3 to 5 bulbs in a meat chopper and straining the mixture through cheesecloth. For testing the volatile substances, onion juice was diluted with distilled water to the following percentages: 50, 40, 32, 25, 20, 15, 10, and 5. A filter paper, 9 cm. in diameter, was placed in the bottom of a petri dish and moistened. On this was placed a U-shaped glass tube supporting 2 glass slides. One of the slides was of the standard size (2.5×7.5 cm.); the other was 1×7.5 cm. The center of the narrow slide was marked and a drop of the concentrated or diluted onion juice placed at this mark. Two drops of spore suspension were placed on the large slide 3 cm. apart and 3 cm. from the drop of onion juice. Thus, the 3 drops formed a triangle with each side measuring 3 cm. The spore suspension was standardized at 20,000 spores per ml. Spores were taken from cultures of *Colletotrichum circinans*, 14 to 21 days old, and spores from cultures of *Botrytis allii*, 7 days old. The drops, both of spore suspension and onion juice, were adjusted as nearly as was possible to the same size. For testing substances in the liquid phase the juice was diluted with distilled water containing spores in suspension to the following percentages; 10, 5, 1, 0.8, 0.6, 0.4, 0.2, and 0.1. Two drops of the material were placed on a slide in a petri dish moist chamber. Controls for each series consisted of drops of spore suspension (spores in sterile, distilled water) placed on slides 3 cm. apart in petri dish moist chambers. The tests were run at room temperature (22° C.±) for 24 hours when from 250 to 500 spores were counted in each drop of spore suspension. Each series was run at least 3 times. If the germination of the controls fell below 95 percent, the series was discarded.

The results of these tests are expressed as that percentage of onion juice at which 50 percent of the spores failed to germinate in 24 hours (LD50). The percentage of germination was computed by first subtracting the percentage of spores not germinated in the control from the percentage not germinated in the tests. The LD50 was arrived at by plotting the corrected percentage of spores not germinated at each dilution on logarithmic probability paper, connecting the points, and from the curve, finding by interpolation or extrapolation the concentration of onion juice at which 50 percent of the spores would not be expected to germinate.

The sulfur content of the fleshy scales was measured by the method described by Currier (*2*).

The effect of dry outer scale extracts was measured by placing 30 disks, cut with a 3-mm. cork borer from the dry outer scales, in 3 ml. of sterile distilled water, allowing them to soak for 2 hours, and then

diluting this extract to the following percentages: 100, 50, 25, and 10. Since it was found by Walker and associates (*17*, *15*) that extracts from the lighter areas of the dry scale were less toxic than those from darker portions, the disks were cut from parts of the scales that were approximately the same in color intensity.

The effect of heat on the toxicity of onion juice was measured by the following procedure. Four milliliters of the extracted juice was placed in each of the desired number of test tubes, and the tubes were placed in constant temperature water baths at 60° and 90° C. The juice was allowed to attain the desired temperature and to remain at that temperature for 7½, 15, 30, and 60 minutes. Upon removal, the juice was immediately cooled to room temperature by insertion in cold water. After all the tubes in a series had been removed and cooled, dilutions were made and the experiment conducted as with unheated juice. The LD50 values for liquid and vapor were computed for each time interval. Controls of spores in distilled water, as well as spores in undiluted unheated onion juice, were set up with each series.

EXPERIMENTAL RESULTS

RELATION OF VARIETY TO SMUDGE INDEX

During the growing seasons of 1944 to 1947, inclusive, a group of onion varieties was grown in replicated, randomized blocks and inoculated with *Colletotrichum circinans*. The varieties were so chosen that white, red, yellow, and brown bulb types were represented. Within the first three color groups, strong and mildly pungent varieties were included. Although a reasonably uniform method of inoculation was followed, the severity of disease development varied with the season.

In table 2, the smudge indices for the four seasons are presented. As a rule, white varieties had very high indices but there were a number

TABLE 2.—*The development of smudge in onion varieties at Madison, Wis., in relation to scale color and pungency*

Scale color	Pungency	Variety	Smudge index in year—			
			1944	1945	1946	1947
White	Mild	White Sweet Spanish	98.7	100.0	88.7	54.5
		Crystal Grano	———	93.6	———	55.5
		Crystal Wax	———	96.7	———	63.5
	Strong	White Portugal	89.3	96.6	51.7	40.9
		Southport White Globe	86.3	93.3	37.3	46.9
		Italian Red	34.7	46.1	15.9	———
Red	Mild	California Early Red	———	48.1	———	50.2
		Lord Howe Island	———	70.4	37.8	27.7
		Creole	———	75.9	38.1	———
	Strong	Red Wethersfield	25.5	40.3	14.7	7.6
		Southport Red Globe	19.5	41.2	———	1.5
Yellow	Mild	Early Grano	88.7	42.9	17.9	19.9
		Yellow Bermuda	78.4	29.9	———	21.0
		Stockton Globe	———	48.2	16.3	———
	Strong	Golden Globe	———	19.5	———	———
		Early Yellow Globe	24.1	25.6	———	———
		Michigan Yellow Globe	———	23.1	———	———
		Brigham Yellow Globe	15.9	44.3	13.5	———
		Yellow Globe Danvers	25.2	29.0	17.6	1.6
		Mountain Danvers	———	34.5	14.7	———
		Ebenezer	16.1	25.8	16.7	2.0
Brown	Strong	Australian Brown	9.7	13.6	25.8	.9
LSD 19:1			11.9	16.3	15.7	8.5
LSD 99:1			15.9	25.4	21.2	11.3

of exceptions. In 1944 and 1945 both strong and mild white varieties had high indices with no significant differences between varieties except that the difference between White Sweet Spanish and Southport White Globe in 1944 was barely significant at the 5-percent level. In 1946, however, when the incidence of smudge as a whole was lower than in the two preceding years the index for White Portugal was highly significantly lower than that of White Sweet Spanish, while that of Southport White Globe was significantly lower than that of White Portugal. In 1947 there was no significant difference between the last two varieties but the index of White Portugal was highly significantly lower than those of the three mild white varieties. That of Southport White Globe was significantly lower than those of two of the three mild varieties. There was in two comparisons, therefore, an obvious inverse correlation between strong pungency and smudge index.

The red varieties as a whole had lower indices than the white varieties. In 1945, however, the indices of two varieties, Lord Howe Island and Creole, were significantly and usually highly significantly greater than those of other red varieties, although they were significantly lower than any white variety in that year. Here there was no inverse correlation of pungency with smudge index, since Creole was strongly pungent and Lord Howe Island was mildly so. In 1946 these two varieties again had indices highly significantly greater than those of other red varieties and not significantly different from those of white strong varieties. The indices were highly significantly lower than that of the white mild variety. In 1947 the index of California Early Red was not significantly different from those of four of the five white varieties and it was highly significantly different from those of pungent red varieties. Lord Howe Island had an index highly significantly less than that of each white variety and of California Early Red, but highly significantly more than those of the pungent red varieties. The indices of California Early Red and Lord Howe Island were significantly in reverse order in 1947 as compared with 1945.

In general, yellow and brown varieties were not significantly different in smudge index from red varieties. The indices of Early Grano and Yellow Bermuda were significantly higher in 1944 and in 1947 than those of other red and yellow varieties. In 1944 the index of Early Grano was not significantly lower than that of any white variety and that of Yellow Bermuda was not significantly lower than those of two of three white varieties. In 1947 the indices of each were highly significantly lower than that of each of five white varieties. In 1945 and 1946 the indices of these two varieties were not consistently higher than those of other red and yellow varieties.

It may be seen from this 4-year survey that in 1944 and 1947 there was a distinct indication of a correlation of mild pungency with higher smudge index in red and yellow groups. In 1945 two red varieties had outstandingly high indices but one was mild and the other strong in pungency. Moreover, in 1946 these same two red varieties had significantly higher indices than did other colored varieties. In 1946 and 1947 the strong white varieties had indices which were highly significantly lower than those of the mild white varieties and again a correlation with mildness and high index was indicated.

Jones et al. (*5*), in a study carried out in 1943, showed that when yellow and white bulb parents which differed only in the color inhibitor gene were crossed, the smudge (as measured by index) in the three color classes (white, cream, and yellow) in the F_2 generation was correlated inversely with the concentration of yellow pigment. Since the inhibitor gene *I* is not completely dominant, the heterozygous individuals (*Ii*) are intermediate in color (cream). In those crosses in which a white, strongly pungent variety was crossed with a yellow, mildly pungent variety the smudge index of the *Ii* class in the F_2 was always slightly higher than the mean of the indices of the *II* (white) and the *ii* (yellow) classes. In the one cross between white, strongly pungent and yellow, strongly pungent varieties the smudge index of *Ii* class in the F_2 generation was lower than the mean of the indices of the white and yellow classes and was, thus, nearer to that of the yellow parent. In all of these crosses the same color gene (*r*) for yellow was present. Since the index for each genotype from strong × strong was distinctly lower than that of the respective color class from strong × mild, the question naturally arose as to whether secondary factors such as those controlling pungency had an effect upon resistance to smudge.

Certain of the F_2 progenies used by Jones et al (*5*) and other closely related ones were included in the smudge tests in 1944 and 1946. The smudge indices of the F_2 progenies and of the parent lines are presented in table 3. The data of the F_2 progenies in 1943 tests were taken from Jones et al. (*5*).

The severity of smudge infection was consistently greater in 1944 than in 1943 and in 1946. When the results from 3 years' tests are considered it may be seen that the indices of the *Ii* class, whether from mild × strong, or strong × strong, were fairly close to the means

TABLE 3.—*The development of smudge in F_2 hybrid progenies from crosses between white, strongly pungent and yellow, mildly pungent and yellow, strongly pungent varieties*

Variety or progeny	Color and pungency	Year of test	Number of progenies	Smudge index in genotype of mean indicated					
				II	*Ii*	*ii*	Mean of *II* and *ii*	Mean of three genotypes	LSD between genotypes at 5-percent level
White Portugal	White, strong	1943	1	50.0					
		1944	1	89.3					
		1946	1	51.7					
Yellow Globe Danvers	Yellow, strong	1943	1			4.5			
		1944	1			29.0			
White Portugal×Yellow Globe Danvers, F_2		1943	21	46.0	18.7	6.0	26.0	23.6	5.6
		1944	4	76.2	47.2	11.7	44.0	45.0	17.3
Early Grano	Yellow, mild	1943	1			53.2			
		1944	1			88.7			
		1946	1			17.9			
White Portugal×Early Grano, F_2		1943	21	72.7	51.1	23.5	48.1	49.1	5.6
		1944	3	94.8	83.0	59.2	77.0	79.0	17.3
Stockton Globe	Yellow, mild	1946	2	63.1	46.5	18.4	40.8	42.7	
		1944	1			48.2			
		1946	1			16.3			
White Portugal×Stockton Globe, F_2		1943	4	88.4	69.9	21.8	55.1	60.0	5.6
		1944	3	98.5	90.1	39.0	68.8	75.9	17.3
		1946	4	58.0	40.3	19.8	38.9	39.3	7.1

between those of the *II* and *ii* genotypes and consequently near to the means of all three classes. It is significant to note, however, that when the yellow, strong variety is compared with that of a yellow, mild variety in any given year the index for the former was distinctly lower. It is also evident, when the means for all genotypes in any given year are compared, that the index was always distinctly lower in the F_2 from white strong × yellow strong than from white strong × yellow mild. This, of course, suggested that strong pungency was a secondary factor influencing resistance to smudge.

RELATION OF VARIETY TO NECK ROT

The creation of a neck rot epidemic by artificial inoculation is not as readily carried out with success as is the case with smudge. Infection is primarily through the maturing neck tissues. Varieties differ in the time at which tops fall over and at which the neck tissues of the bulbs mature. Infection normally takes place just before this period closes if environal conditions are favorable and if inoculum is built up abundantly on sloughed leaves and scales. The dates of inoculation for each year are given in table 1. In 1944, 1945, and 1947 the plants were sprinkled for several hours after each inoculation. In 1946 the overhead sprinklers were run throughout the night four to five nights per week for the period between inoculation and harvest. The results are given in table 4. In 1944, White Sweet Spanish was the latest variety to mature and the high percentage of infected

TABLE 4.—*The development of neck rot in onion varieties at Madison, Wis., in relation to scale color and pungency*

Scale color	Pungency	Variety	Neck rot in year—			
			1944	1945	1946	1947
			Percent	*Percent*	*Percent*	*Percent*
White	Mild	White Sweet Spanish	84.2	3.5	41.9	4.80
		Crystal Grano		.8		2.8
	Strong	Crystal Wax		25.1		33.5
		White Portugal	.04	.7	16.3	3.1
		Southport White Globe	1.7	.9	19.0	3.1
		Italian Red	16.4	1.4	38.9	
Red	Mild	California Early Red		5.4		37.0
		Lord Howe Island		2.0	33.6	30.3
	Strong	Creole		0	5.7	
		Red Wethersfield	11.1	0	17.0	3.1
		Southport Red Globe	6.7	0		4.5
Yellow	Mild	Utah Sweet Spanish			23.7	4.2
		Early Grano	0	5.8	42.8	22.0
		Yellow Bermuda	0	5.9		16.7
		Stockton Globe		.8	16.2	
	Strong	Golden Globe		.7		
		Early Yellow Globe	5.2	.8		
		Michigan Yellow Globe		0		
		Brigham Yellow Globe	3.9	0	18.8	
		Yellow Globe Danvers	.6	0	8.1	3.1
		Mountain Danvers		.8	8.1	
		Ebenezer	4.6	0	5.8	3.3
Brown	Strong	Australian Brown	0	.8	3.4	2.0
Average of all white varieties					25.7	9.5
Average of all red varieties					23.8	19.0
Average of all yellow varieties					21.9	9.9
Average of all mild varieties					32.9	18.9
Average of all strong varieties					14.2	3.2
LSD 19:1					18.6	[1] 17.9
LSD 99:1					25.1	[1] 25.1

[1] Analysis of variance of 1947 data applies only to the following varieties: Crystal Wax, California Early Red, Lord Howe Island, Early Grano, and Yellow Bermuda.

bulbs showed that inoculation was timely for this variety. In 1945 only Crystal Wax, an early maturing variety, showed an appreciable percentage of infected bulbs. In 1946 when infection was more general than in other years, the greatest difference was not between white and colored varieties as has been usually reported previously (*15*, *16*, *17*), but between mild and strong varieties. In any color class the mild varieties had significantly higher percentages of bulbs affected with neck rot than strong varieties. The only exception was Stockton Globe. The variety averages for each color class were very close. When all mild varieties were placed in one group and all strong varieties in another, there was a wide and undoubtedly significant difference. It was evident that when conditions were continuously favorable for neck rot there was an inverse correlation between pungency and disease development. In 1947 the incidence of disease was, in general, relatively low. When it did appear in fairly large amounts, however, it was again always in mild varieties about evenly distributed between the white, yellow, and red groups.

RELATION OF VARIETY TO BLACK MOLD

In 1944, natural infection with black mold indicated that colored varieties were more susceptible than white varieties. Artificial inoculation was made just before harvest in 1945, and abundant infection was found after harvest. In 1946, an earlier inoculation was made and it was assumed that the frequent overhead irrigation practiced would be sufficient to provide subsequent inoculum. Apparently this was not the case since very little black mold developed in 1946. In 1947, when frequent overhead irrigation was not practiced except for a 2-hour period after inoculation, black mold was again quite prevalent. The best evaluation of varieties can be made, therefore, from the 1945 and 1947 experiments (table 5). In 1945 all colored varieties were more

TABLE 5.—*The development of black mold in relation to scale color and pungency in onion varieties at Madison, Wis.*

Scale color	Pungency	Variety	Black mold in year—				
			1944	1945	1946	1947	1947
			Percent	*Percent*	*Percent*	*Percent*	*Index*
White	Mild	White Sweet Spanish	0	0	0	13.7	6.0
		Crystal Grano		8.0		22.5	11.0
		Crystal Wax		5.3		51.5	28.9
	Strong	White Portugal		2.9	.7	42.9	19.2
		Southport White Globe	.8	1.3	0	66.5	31.9
Red	Mild	Italian Red	63.8	24.3	0		
		California Early Red		29.3		66.7	28.3
		Lord Howe Island		39.3	0	55.8	23.5
	Strong	Creole		27.1	3.5		
		Red Wethersfield	16.0	34.4	0	62.4	28.1
		Southport Red Globe	8.0	33.8		60.3	24.2
		Utah Sweet Spanish			.8	20.1	9.1
Yellow	Mild	Early Grano	0	32.6	0	39.2	13.8
		Yellow Bermuda	1.1	35.0		71.6	38.3
		Stockton Globe		36.5	0		
	Strong	Golden Globe		37.1			
		Early Yellow Globe	9.4	45.6			
		Michigan Yellow Globe		42.1			
		Brigham Yellow Globe	9.3	48.7	.7		
		Yellow Globe Danvers	14.4	47.1	2.2	62.6	25.7
		Mountain Danvers		69.8	0		
Brown	Strong	Ebenezer	12.6	60.4	0	78.3	32.3
		Australian Brown	25.8	77.1	2.0	78.9	41.3
LSD 19:1							8.2
LSD 99:1							10.9

severely affected than white varieties. Within color groups there was no evidence of correlation between pungency and the percentage of infected bulbs. Australian Brown, Mountain Danvers, and Ebenezer were outstanding in the high percentage of infected bulbs. In 1947 there was not a distinct correlation between color and incidence of black mold, nor was there any correlation between pungency and disease incidence. When the bulbs were placed in four classes and an index for each lot determined the same relation between varieties was maintained.

RELATION OF ANTIBIOTIC COMPONENTS AND VOLATILE SULFUR CONTENTS OF FLESHY ONION TISSUE TO DISEASE INDEX

One of the principal purposes of the investigation was to determine whether antibiotic components of the fleshy tissue of onion varied with variety and whether they in any way influenced the resistance to smudge brought about by the soluble phenolic antibiotics in the dry outer colored scales. On the basis of results already presented in table 2 it was evident that there was not a consistent correlation between pungency and resistance. The question was studied further by determining the toxicity of the expressed onion juice and of the volatile vapor arising from it by methods already described. These tests were made with representative bulbs from the disease plots of 1944 and 1945. In 1945 the volatile sulfur was determined. The results are presented in table 6.

TABLE 6.—*The volatile and nonvolatile antibiotic components of succulent onion scale tissue as measured by the LD50 values for* Colletotrichum circinans *in relation to scale color, pungency, and volatile sulfur content*

Scale color	Pungency	Variety	LD50 value of—				Volatile sulfur 1945
			Volatile component		Nonvolatile component		
			1944	1945	1944	1945	
			Percent	*Percent*	*Percent*	*Percent*	*Mg. per 10 gm.*
White	Mild	White Sweet Spanish	59.1	64.3	0.87	0.91	13
		Crystal Grano		43.3		.52	22
		Crystal Wax		42.0		.49	18
	Strong	White Portugal	16.8	13.3	.69	.55	25
		Southport White Globe	21.3	18.3	.71	.56	24
		Italian Red	17.3	12.3	.71	.60	12
Red	Mild	California Early Red		81.3		.90	13
		Lord Howe Island		49.0		.73	11
	Strong	Creole		19.3		.57	21
		Red Wethersfield	21.0	29.0	.66	.50	24
		Southport Red Globe	18.9	17.6		.56	21
Yellow	Mild	Early Grano	42.9	44.6	.73	.32	11
		Yellow Bermuda	32.7	29.6	.66	.58	14
		Stockton Globe		40.3		.99	13
	Strong	Golden Globe		22.6		.50	15
		Early Yellow Globe	27.3	31.3	.78	.72	14
		Michigan Yellow Globe		42.6		.36	14
		Brigham Yellow Globe	27.8	21.6	.38	.44	13
		Yellow Globe Danvers	22.0	17.6	.63	.59	13
		Mountain Danvers		28.3		.97	14
		Ebenezer	36.3	47.6	.49	.60	16
Brown	Strong	Australian Brown	5.1	9.0	.78	.99	21
LSD 19:1				2.8		.09	
LSD 99:1				3.7		.12	

With some notable exceptions the volatile components from mild varieties were usually significantly less toxic to *C. circinans* than those from strong varieties. Among white varieties this was consistently true in 1944 and 1945. In red varieties it was also true with the conspicuous exception of Italian Red. In yellow varieties it was not consistently true. Austrialian Brown, which is a very pungent variety, had the most toxic volatile component. When the liquid was used as the test fluid by adding spores directly to it, it is possible that some of the volatile component was still present. There was, however, no consistent correlation between the LD50 values of the liquid (nonvolatile) and the volatile components. There was no correlation between toxicity of the liquid and pungency, the LD50 of Australian Brown being, for instance, not significantly different from that of White Sweet Spanish in 1945. The volatile sulfur content of the juice bore no relation to the toxicity of the volatile component. This indicated strongly that allyl disulfide or other volatile sulfur compounds were not the important toxic constituents of the volatile component.

In 1944, the segregates from crosses between white strong and yellow strong and between white strong and yellow mild varieties were tested for smudge resistance (table 3). It was shown in the first case that the smudge index of the white segregates was close to that of the white parent and that of the yellow segregates close to that of the yellow parent, while that of the heterozygotes, intermediate in color, was near the mean of the first two classes. In the second cross each parental line had a high smudge index. The unexpected high index of this yellow parent was probably due to its poor outer scale protection, a matter to be discussed in a later section of this paper. The segregates in the F_2 generation of this class reacted differently than those of the white strong × yellow strong cross. The index of the white segregates was slightly above that of the white parent, the index of the yellow segregates (50.2) was lower than that of the white segregates but significantly higher than that of the yellow segregates in the first cross. The index of the heterozygote class was not significantly different from that of the two parents. It was of interest to compare the toxicity of the volatile component of the color segregate groups in the F_2 generation of the two crosses. The results together with the smudge indices are given in table 7. In the White Portugal × Yellow Globe Danvers group all volatile components were rather

TABLE 7.—*Relation of volatile antibiotic components to scale color in F_2 segregating progenies from crosses between white strongly pungent, yellow strongly pungent, and yellow mildly pungent varieties of onion*

Variety or progeny	Color pungency	Genotype	Smudge index	LD50 of volatile component
White Portugal	White, strong	II	89.3	16.8
Yellow Globe Danvers	Yellow, strong	ii	29.0	25.2
White Portugal × Yellow Globe Danvers—F_2		II	76.2	17.5
		Ii	47.2	11.0
		ii	11.7	6.3
Early Grano	Yellow, mild	ii	88.7	42.9
White Portugal × Early Grano—F_2		II	94.8	32.0
		Ii	83.0	40.3
		ii	59.2	9.0
LSD 19:1			17.3	

high and the LD50 of the F_2 segregates was about as low as or lower than either parent. There was no relation of toxicity to smudge index. In the White Portugal × Early Grano cross the white and intermediate color classes had high LD50 values and thus low toxicity, while the yellow segregates had an LD50 value slightly lower than that of the strong (white) parent. Again in this cross there appeared to be no relation between smudge index and the antibiotic activity of the volatile component.

The smudge and the black mold organisms first relate themselves to the onion bulb by growing saprophytically on the dead outer bulb scales. Although they can infect the living tissue of the fleshy scales their progress as disease incitants is usually very slow. The neck rot organism, *B. allii*, on the other hand, after it has penetrated the fleshy tissue, usually through the neck of the bulb, is a rapid rot-producing pathogen. It was postulated by Walker et al. (*17*, *18*) that the antibiotic components of the fleshy scales were a factor in determining the relative rate of decay by these three organisms. It has already been shown in this investigation (table 4) that the mild varieties of onion showed a higher percentage of infection by *B. allii* than did strongly pungent varieties. Strong and mild varieties were selected and LD50 values were determined for the juice and the volatile component of the juice for each. The value in each case was secured for *C. circinans*, *A. niger*, and *B. allii*. The results are presented in table 8.

TABLE 8.—*The sensitivity of Colletotrichum circinans, Botrytis allii, and Aspergillus niger to the volatile and nonvolatile antibiotic components of onion in relation to scale color and pungency*

Scale color	Pungency	Variety	LD50 value of—					
			Volatile component on—			Liquid component on—		
			C. circinans	*A. niger*	*B. allii*	*C. circinans*	*A. niger*	*B. allii*
White	Mild	White Sweet Spanish	64.3	97.1	180.0	0.91	0.61	111.0
	Strong	White Portugal	13.3	55.4	112.0	.55	.43	99.9
Red	Mild	California Early Red	81.3	109.3	180.0	.90	.59	143.0
	Strong	Southport Red Globe	17.6	61.1	130.0	.56	.51	101.0
Yellow	Strong	Yellow Globe Danvers	17.6	78.3	110.0	.59	.55	128.0
	Strong	Ebenezer	47.6	92.7	155.0	.60	.50	96.3

It will be seen that volatile components of the two mild varieties, White Sweet Spanish and California Early Red, had the highest values and thus the lowest toxicity for each of the three organisms. This was also true for liquid extract. It is also clearly evident that for both the volatile and the liquid components of the varieties as a whole, *C. circinans* and *A. niger* were much more sensitive than *B. allii*. While these facts cannot be put forth as final proof of the relation of the antibiotic substances in onion tissue to the rate of disease progression by the three organisms, there seems to be little doubt that the hypothesis is a plausible one. Undoubtedly other factors enter into the host-parasite interactions, but until contrary evidence is forthcoming it is justifiable to conclude that the smudge and black mold are retarded in the fleshy tissue by toxic components of the tissue other than the nonvolatile phenolic compounds found in the dry outer scales of

colored varieties. Moreover, the fleshy scale antibiotics probably are a factor in reducing the amount of neck rot infection in strongly pungent as compared with mild varieties.

It was suggested further by Walker et al. (*18*) that substances secreted by the smudge organism after infection might attenuate these antibiotic substances in the tissue and facilitate progress of the pathogen. To test this hypothesis tissue was obtained from diseased bulbs by cutting 3-mm. plugs with a cork borer. The areas selected were those in which the fleshy scale was unaffected and in which the same scale exhibited a typical yellow, wrinkled appearance immediately below an outer scale lesion. The plugs were placed on glass slides and crushed, the same crushing pressure being used in each test. Drops of spore suspension were placed at 0.5-cm. intervals away from the crushed tissue since it had been found that germination of spores in water varied in proportion to the distance the drops of spore suspension were from the drops of onion juice in the moist chamber. The averages of nine replicates are presented in table 9. It is evident that

TABLE 9.—*The relative antibiotic activity of the volatile component of fleshy scale tissue infected by and of that not infected by Colletotrichum circinans*

Variety	Infected or noninfected tissue as source of antibiotic	Percentage of spores of *C. circinans* germinated at the distance indicated from the source of the volatile antibiotic				
		2 cm.	2.5 cm.	3 cm.	3.5 cm.	4 cm.
White Portugal	Infected	0	46	61	83	94
	Noninfected	0	8	31	51	88
Ebenezer	Infected	0	32	74	91	97
	Noninfected	0	18	43	77	91

the volatile component from the noninfected tissue was more toxic than that from infected tissue.

Another type of experiment was performed to determine whether *C. circinans* produced any substance which inactivated the antibiotic substances in the fleshy scale. A culture of the fungus was grown on a modification of Duggar's medium [3] for 30 days. After spores and mycelium were removed, the filtrate was used as a diluent of the juice extracted from fleshy onion scale tissue. A portion of the original medium was used as a control diluent. After the dilutions were made the mixtures were allowed to stand for 2 hours and spores of *C. circinans*, were introduced. The LD50 values determined 24 hours later are given in table 10. They show that the filtrate from the fungus culture attenuated the antibiotic substance contained in the fleshy scale extract.

RELATION OF OUTER SCALE EXTRACT TO BLACK MOLD

It has been shown in table 5 that in one year colored varieties were much more susceptible to black mold than were white varieties and, in general, colored varieties were not resistant to black mold to the

[3] 25 ml. 10 percent dextrose; 10.0 ml. 0.5M KNO_3; 10 ml. 0.20M KH_2PO_4; 5.0 ml. 0.10M $MgSO_4$; 0.5 ml. 0.001M $FeCl_3$; 10 ml. of a minor element solution consisting of 2.818 gm. H_3BO_4, 0.04 gm. $CuCl_2.2H_2O$, 0.03 gm. $ZnCl_2$, and 0.39 gm. $MnCl_2$ per liter; made up to 1,000 ml. with distilled water.

TABLE 10.—*Effect of filtrate from a culture of Colletotrichum circinans in liquid medium on the antibiotic activity of fleshy onion scale extract*

Material tested	LD50 value of—	
	Volatile component	Nonvolatile component
Onion juice diluted with uninoculated liquid medium	42.0	0.60
Onion juice diluted with filtrate from culture of C. circinans	120.0	.95

degree that they were to smudge. It is also evident from the data presented in table 8 that *A. niger* was much more sensitive than *B. allii* to the volatile and nonvolatile antibiotic substances of the fleshy scales of both colored and uncolored bulb varieties. Since the black mold organism develops primarily on the dry outer scales of onion bulbs, it is obvious that the antibiotic substances in these scales are of more importance than those of the fleshy scales. Extracts from white, red, yellow, and brown outer scales were made by methods already described. Each extract was diluted to various degrees with distilled water and germination tests with spores of *A. niger* were made. *C. circinans* was also tested in the undiluted extract. The results are presented in table 11.

TABLE 11.—*Effects of extracts of outer colored and white scales upon the germination of Aspergillus niger and Colletotrichum circinans*

Dilution in distilled water	Organism	Percentage of spores germinating in scale extract indicated				
		White	Red	Yellow	Brown	Water control
None	*C. circinans*	98	0	0	0	98
None	*A. niger*	10	100	100	100	0
3 to 1	do	0	85	100	100	0
1 to 1	do	0	69	100	90	0
1 to 3	do	0	29	83	71	0
1 to 10	do	0	0	4	0	0

C. circinans, as already pointed out by Walker (*14*), germinated well in distilled water and in white scale extract but was completely inhibited by colored scale extract. *A. niger* does not germinate in distilled water but does so readily if a favorable nutrient is in the liquid. When exposed to undiluted scale extracts, the two organisms reacted in reverse order. There was slight germination in undiluted white extract and complete germination in undiluted extracts from each type of colored scale. As the extracts were diluted, the percentage of germination dropped, indicating that nutrient or stimulative materials became less effective with dilution. White-scale extract stimulated no germination at 3 to 1. At 10 to 1, no germination occurred in red- and brown- and only 4 percent in yellow-scale extract. It is evident that the materials conducive to germination of *C. circinans* in white scales do not stimulate *A. niger*, while the materials in colored scales toxic to *C. circinans* are stimulative to *A. niger*. Walker and Link (*19*) found that protocatechuic acid had no depressive effect on *A. niger* and that the latter organism was much less sensitive to catechol than

C. circinans and *B. allii*, while a closely related compound, gallic acid, was stimulative to *A. niger*. It is not unlikely that the same phenolic compounds which provide resistance to smudge in colored varieties may be the materials which favor susceptibility to black mold in the same varieties.

NATURE OF THE ANTIBIOTICS

Walker, Lindegren, and Bachmann (*18*) found that the toxic materials in onion juice were of at least two kinds. One was volatile and thus quite easily driven off by heating for 15 minutes; the other was stable and remained toxic after 90 minutes at 90° C. This study was repeated and the effect of certain adsorptive agents on the antibiotics was tested. Portions of onion juice were agitated with 10 percent, by weight, of the material and filtered through an asbestos mat. The filtrate was tested against *C. circinans* and the LD50 values for vapor and for liquid were obtained.

The temperature experiments (table 12) confirmed the work of Walker et al. (*18*). The abrupt change in the LD50 of the volatile

TABLE 12.—*The effect of temperature on the antibiotic value of juice from succulent onion scales*

Variety	Phase	Temperature	LD50 of juice after time (minutes) of exposure indicated				
			0	7½	15	30	60
		°C.					
Southport White Globe	Vapor	60	23	95	[1]109		
	do	90		93	112		
	Liquid	60	.3	.9	.96	0.99	1.4
	do	90		.5	7.5	8.5	8.5
White Sweet Spanish	Vapor	60	18	104	111		
	do	90		110	124		
	Liquid	60	.5	.9	1.1	2.0	3.5
	do	90		4.1	6.2	7.8	8.0
Yellow Globe Danvers	Vapor	60	18	120	134		
	do	90		121	142		
	Liquid	60	.8	4.0	4.0	4.9	5.0
	do	90		5.1	7.5	7.5	7.5
California Early Red	Vapor	90	55	146	157		
	Liquid	90	.4	4.5	7.5	9.5	9.5

[1] Values above 100 were determined by extrapolation, as explained under Materials and Methods.

material after 7½ minutes in contrast with the slight change in the LD50 of the liquid component showed that the original toxic material in the macerated fleshy scale tissue is composed of a thermolabile and a thermostable fraction. The LD50 of the liquid phase changed little after the initial rise which was probably caused by the loss of the volatile antibiotic. After heating the liquid phase for 30 minutes no volatile toxins could be detected. The fact that the LD50 of the liquid component was higher after heating for 60 minutes at 90° than for the same period at 60° suggests that the portion remaining after the toxins were driven off or decomposed might also have consisted of two parts, the smaller part being inactivated at the higher temperature, or that the same inactivation process continued over the 60-minute interval.

The results of the tests with adsorptive agents are presented in table 13. Super Cel removed some of the volatile antibiotic but none of that in the liquid phase. Celite removed slightly more of the antibiotic in each phase. Nuchar W treatment resulted in an LD50 for the

TABLE 13.—*The effect of various materials on the toxicity of juice from Southport White Globe*

Material	Phase	LD50
Asbestos	Vapor	24
	Liquid	.78
Asbestos and Super Cel	Vapor	76
	Liquid	.89
Asbestos and Celite	Vapor	95
	Liquid	4.0
Asbestos and Nuchar W	Vapor	115
	Liquid	45.0
Control	Vapor	20.5
	Liquid	.3

volatile component practically the same as the maximum secured by heating. When the liquid phase was treated with Nuchar W, the LD50 was distinctly higher than that of heated juice (45 as opposed to 8.5). This showed that all of the antibiotic in the liquid was not destroyed by heat.

Equal parts of onion juice and ether were shaken together and the ether-soluble fraction tested after the ether had evaporated off. The LD50 was 23. The LD50 of the residue was 34. A small portion of antibiotic component was apparently soluble in ether.

RELATION OF OUTER SCALE CHARACTER TO SMUDGE INDEX

Inasmuch as the evidence had gradually accumulated that the wide differences which sometimes occurred in smudge indices within colored variety groups were not consistently related to the antibiotic components of the fleshy tissue, other characters were sought which might explain the differences. It may well be emphasized here that it was early observed by Walker (*12*, *14*, *15*) that the dry outer scales of resistant colored bulb varieties held the key to resistance and that when such colored scales were removed the fleshy colored scales were infected as readily as succulent uncolored scales. Moreover, when the phenolic compounds, protocatechuic acid and catechol, were isolated later (*6*, *8*), it was emphasized that they functioned because they were water-soluble and diffused easily into the infection drop present on outer dry scales but that they could not do so readily from the epidermal cells of succulent scales. The outer scale of the colored bulb, therefore, protects the underlying fleshy scale from infection.

In the current study of onion varieties it was noted that they differed widely in the number of outer scales which normally occurred and in the completeness with which they covered the fleshy scales. This degree of protection also varied somewhat from season to season. Beginning in 1945, this character was noted at harvest and later in storage and the bulbs of each variety were placed as a group in 1 of 3 classes already described, i. e., good, poor, very poor. In table 14 are given the scale ratings of each variety tested, together with the smudge indices for each year.

There was no relation between scale development and smudge index on white varieties, as may have been expected. In the colored varieties, however, significant correlation occurred. In 1945 all red varieties were poor or very poor and the indices were all higher than those of yellow varieties rated as good. Within the yellow varieties the three with poor scales had the highest indices. In the red group, Lord Howe

TABLE 14.—*Relation of smudge index to outer scale development in various onion varieties*

Scale color	Pungency	Variety	1945		1946		1947	
			Scale[1] development	Smudge index	Scale[1] development	Smudge index	Scale[1] development	Smudge index
White	Mild	White Sweet Spanish	G	100.0	G	88.7	G	54.5
		Crystal Grano	P	93.6			G	55.5
		Crystal Wax	P	96.7			G	63.5
	Strong	White Portugal	G	96.6	G	51.7	G	40.9
		Southport White Globe	G	93.3	G	37.3	G	46.9
		Italian Red	P	46.1	P	15.9		
Red	Mild	California Early Red	P	48.1			VP	50.2
		Lord Howe Island	VP	70.4	VP	37.8	VP	27.7
	Strong	Creole	VP	75.9	VP	38.1		
		Red Wethersfield	P	40.3	G	14.7	G	7.6
		Southport Red Globe	P	41.2			G	1.5
Yellow	Mild	Early Grano	P	42.9	P	17.9	P	19.9
		Yellow Bermuda	G	29.9			P	21.0
		Stockton Globe	P	48.2	G	16.3		
	Strong	Golden Globe	G	19.5				
		Early Yellow Globe	G	25.6				
		Michigan Yellow Globe	G	23.1				
		Brigham Yellow Globe	P	44.3	G	13.5		
		Yellow Globe Danvers	G	29.0	G	17.6	G	1.6
		Mountain Danvers	G	34.5	G	14.7		
		Ebenezer	G	25.8	G	16.7	G	2.0
Brown	Strong	Australian Brown	G	13.6	G	25.8	G	.9
LSD 19:1				16.3		15.7		8.5
LSD 99:1				25.1		21.2		11.3

[1] G=good; P=poor; VP=very poor.

Island and Creole had very poor scale development and the smudge index of each of these was significantly greater than that of any other colored variety. In 1946 the same two varieties had significantly higher smudge indices than other colored varieties and again they were very poor in scale development. Yellow varieties rated poor in 1945 were, with the exception of Early Grano, not so rated in 1946 and, correspondingly, the smudge indices were not significantly different from those of other colored varieties with good scale development. In 1947, the yellow and red varieties with poor outer scales had significantly higher indices than other colored varieties. It appears, therefore, that this scale character is an important secondary factor in determining smudge resistance. When fleshy scales are poorly protected the outermost fleshy scale becomes infected before harvest and the disease progresses in storage about as well as in a white variety. Smudge development in the fleshy scale of a colored variety, exposed by sloughing of the outer scale, is shown in figure 1.

DISCUSSION

The early work on resistance of onions to smudge led to the discovery that certain chemicals, protocatechuic acid and catechol, present in the dry outer scales of colored onions were toxic to *Colletotrichum circinans*. This was the first case in which it was proved that chemicals, secreted by the host, were responsible for resistance to a disease of that host. In later work concerning the method of inheritance of color it was found that yellow color in various varieties was controlled by the same gene. The indices of varieties having the same color, however, sometimes differed widely, as did the indices of progenies from crosses between white and colored varieties. These discrepancies led to the assumption that secondary characters influ-

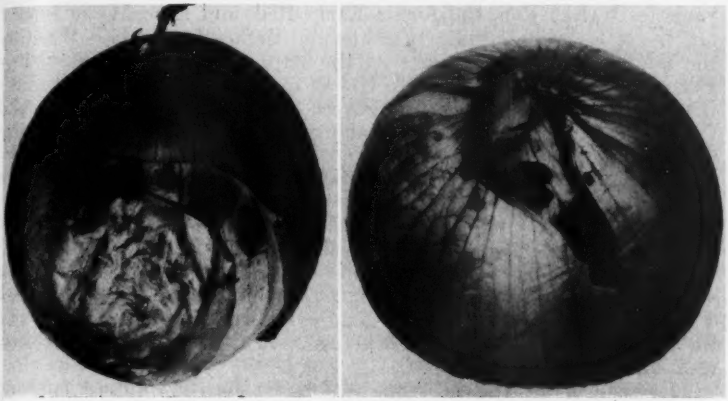

FIGURE 1.—Development of smudge on the fleshy scale of a yellow variety, Early Grano, in which the protective fungicidal effect of soluble phenolic antibiotics in the dry outer scales ordinarily functions as the resistant character but which has failed in this case because of the tendency in this variety for the outer scales to slough readily or split so as to expose the vulnerable fleshy scale.

enced resistance. This seemed especially plausible because of the fact that the juice from succulent scales of onion had bactericidal and fungicidal properties. Walker and associates (*18*) found that *C. circinans*, *Aspergillus niger*, and *Botrytis allii* were susceptible to the vapor given off by crushed onion tissue as well as to the liquid from the crushed scales. However, the fact that the bulb was parasitized by these organisms suggested that an attenuation of toxic materials occurred and that the degree of infection, governed primarily by the presence or absence of phenolic compounds associated with color, was secondarily affected by the tolerance of the fungi to the fungicidal properties of the onion juice.

Comparative trials during four successive seasons showed that mild varieties usually had higher smudge indices than strong varieties. There were, however, some outstanding exceptions which indicated rather definitely that other factors than pungency were involved. When the volatile antibiotics were assayed there was an inverse correlation between toxicity and smudge index in white varieties and in all cases but one with red varieties; but in yellow varieties there was no clear-cut correlation. There was no correlation between the toxicity of volatile substances and their sulfur content and none between the volatile substance and the toxicity of the nonvolatile antibiotics in the succulent scales toward smudge organism. The wide differences in smudge indices of varieties within color groups were, therefore, not consistently associated with the antibiotics in succulent scales. A much more plausible explanation of the varietal differences was found in the physical nature of the protective dry outer scales. Where colored varieties showed high smudge indices the dry outer scales were usually limited to one or two in number and sloughed readily or split so as to expose the vulnerable fleshy scales to the organism. This character appeared to be the most important in predisposing colored varieties to infection and offered a plausible explanation for the high incidence of smudge in some years in the red

varieties Italian Red, California Early Red, and Lord Howe Island, and the yellow varieties, Early Grano and Yellow Bermuda. Whether the tendency in some seasons of the strong white varieties, White Portugal and Southport White Globe, to have lower smudge indices than the mild varieties, White Sweet Spanish, Crystal Grano, and Crystal Wax, is due to the greater toxicity of volatile antibiotics in the strongly pungent varieties has not been answered by these studies.

In the case of neck rot a satisfactory epidemic was produced only in 1946. In this case there was greater correlation between mild pungency and disease incidence. In 1947 those varieties in which 16 percent or more of the bulbs were infected were all mildly pungent. Both the volatile and nonvolatile antibiotics of the mild varieties tested were less toxic to *Botrytis allii* than those of the strong varieties tested. There appears to be reasonably good evidence that the antibiotics in the fleshy scales have a direct bearing upon the response of varieties to *B. allii*.

The black mold disease is one which is concerned almost entirely with the dry outer scale tissue. Certain colored varieties, especially Australian Brown, are rather consistently susceptible to this disease both in the field generally and in the inoculation plots in these investigations. In one season the colored varieties were consistently higher in disease incidence than white varieties. A comparison of extracts of colored and white outer scales showed that, in the former, germination was stimulated while in the latter it was not. This is interpreted as a difference in lack of essential nutrients in white onion scales rather than the presence of an antibiotic. On the other hand, there is good circumstantial evidence that the phenolic substances in dry outer colored scales, which are so antibiotic to the smudge and neck rot organisms and constitute the major factor in resistance to them, are the factors which favor susceptibility to black mold in the same varieties.

The degree of parasitism of *C. circinans*, *B. allii*, and *A. niger* on succulent scales after infection of the latter by circumvention of the colored outer scales in one way or another in the case of the first two organisms and by encouragement of the phenolic compounds associated with color in the case of the third organism, is an important and interesting question. It is quite obviously not concerned primarily with varietal resistance discussed above, except possibly in the case of neck rot. *B. allii* is the most aggressive rot producer once it has gained a foothold, *C. circinans* is the next most aggressive but much less so. *A. niger* is little more than a superficial saprophyte growing profusely on the dead tissue of dry outer scales and between them and the first fleshy scale but scarcely invading the sound succulent tissue.

It was suggested earlier by Walker et al. (*18*) that the volatile and/or nonvolatile antibiotics in succulent tissue might have a bearing upon the relative success of these organisms as pathogens. Of course, one cannot overlook the difference between these organisms in their inherent enzymic or other mechanisms for penetration and parasitic action. In fact, it is so far extremely difficult to distinguish between such offensive properties of the organisms and the defensive properties of the host substrate. By the methods used herein, however, there is a means of evaluating the antibiotic effect of volatile and nonvolatile

constituents of the succulent onion tissue. The data in table 8 show that *A. niger* is much more sensitive than *B. allii* and nearly as sensitive as *C. circinans* to the volatile antibiotics; what is probably still more important is that it is more sensitive than either one of the antibiotics in the liquid phase. Its reaction to the antibiotics in the fleshy tissue is directly opposite to its reaction to the phenolic antibiotics of the outer scales of the colored varieties, to which *C. circinans* and *B. allii* are both extremely sensitive. There is, therefore, a fairly clear inverse correlation between the aggressiveness of these three organisms as decay producers after infection of the succulent scales and their sensitivity to the antibiotics in those succulent scales. There is, moreover, an inverse correlation, but one of a reverse order, between their aggressiveness as to establishment on the dry outer scales and their sensitivity to the water-soluble phenolic antibiotics in those scales. There are, in other words, two groups of antibiotics here which have a major influence upon the response of varieties to these organisms and upon the pathogenic aggressiveness of the organisms in the fleshy scales.

Colored varieties are more susceptible to black mold because the substances in the dry outer scales appear to favor the development of the organism as a saprophyte on those scales. The extreme sensitivity of this organism to the volatile and nonvolatile antibiotics in the succulent scales of both white and colored varieties appears to be a deterrent factor in restricting the organism to a superficial and extremely mild pathogenesis.

The smudge organism is restricted to white varieties by its extreme sensitivity to the phenol antibiotics in dry outer scales of colored varieties except in those cases where the outer scales slough or split so as to expose the fleshy scales. After infection of the succulent scales of colored or uncolored varieties its progress is impeded by the volatile and nonvolatile antibiotics in the succulent tissue which appear to restrict its pathogenic action, but there is evidence that the fungus neutralizes these antibiotics to some extent.

The neck rot organism, being primarily a wound invader, gains access usually at the neck of the bulb. It is sensitive to the phenol antibiotics in the outer scales, which have a partially restraining effect although they are inclined to be less uniformly prevalent in the neck tissues than in the bulb proper. In many instances they do provide the resistant barrier commonly associated with colored varieties in relation to this disease. If the fungus becomes established in succulent tissue, however, resistance in relation to color no longer holds. The fungus is the least sensitive, of the three considered here, to the volatile and nonvolatile antibiotics of the succulent scales and this is probably a factor in determination of the fact that it is the most effective decay producer of the three. This factor probably is a dominant one also in the greater susceptibility of mild varieties, as compared with pungent ones, to neck rot.

SUMMARY

Varieties of onion representing white, red, yellow, and brown groups, and the mild and strongly pungent types within the first three groups, were tested during four seasons (1944–47) for resistance under field conditions to smudge (*Colletotrichum circinans*), neck rot (*Botrytis allii*), and black mold (*Aspergillus niger*).

While colored varieties were in general more resistant to smudge than white varieties there were some notable and rather consistent exceptions. Strongly pungent varieties in red, yellow, and white groups tended in some seasons to have significantly lower smudge indices than mildly pungent varieties. In F_2 progenies from crosses between white strong × yellow mild and between white strong × yellow strong the heterozygous class from the latter cross had a consistently lower smudge index. These facts indicated that pungency had an effect upon varietal resistance to smudge, but it did not explain some outstanding cases where a colored strong variety had a smudge index not significantly different from those of mild varieties.

Bioassays of the volatile and nonvolatile antibiotic substances in the fleshy scales of the varieties failed to show a definite correlation between toxicity and smudge index. There was no correlation between content of volatile sulfur and pungency and none between sulfur and toxicity of the volatile antibiotics.

The character of the outer scales of varieties varied greatly in number and in tendency to slough and to split so as to expose the fleshy scale. In those colored varieties which had only one or two easily sloughing outer scales, which also commonly split before sloughing, infection of the fleshy scales took place readily. The "poor outer scale" character varied somewhat from year to year in the same variety but in colored varieties it was correlated with high smudge index. This outer scale character is regarded as the most important one modifying the smudge resistance of colored varieties which is due primarily to the phenolic antibiotics in the outer scales.

In the one year in which neck rot developed severely enough to evaluate the resistance of varieties there was no correlation between resistance and color, but a distinct correlatiton between strong pungency and resistance. There was also a positive correlation between resistance and the toxicity of the volatile and nonvolatile antibiotics in fleshy scales.

Black mold was in one season significantly more severe on colored than on white varieties. Australian Brown, the most deeply colored variety, had the highest percentage of infected bulbs in two out of the three seasons in which the disease was quite extensive. The extract of dry outer colored scales was distinctly stimulative to germination and growth of the black mold organism, when compared with white outer scale extract. It appears that the same phenolic substances which are the major factors for resistance to smudge and which play a part in neck rot resistance may be the ones which contribute to black mold susceptibility in colored varieties.

When each of the three organisms gains access to the fleshy scales, there is no correlation between color and resistance, but the three species represent distinctly different degrees of pathogenicity. *A. niger* is very mildly pathogenic and is little more than a saprophyte on dry outer scales. *C. circinans* is somewhat more aggressive in fleshy scale decay but is still a relatively slow invader. *B. allii* is an aggressive fairly rapid rot producer. When the three organisms were compared as to their sensitivity to the nonvolatile antibiotics in the fleshy scales they assumed a descending scale of sensitivity in the order mentioned, although the differences between the first two were sometimes small. In the case of the volatile antibiotics the order of *C. circinans* and

A. niger was reversed, but *B. allii* was again the least sensitive. It appears that the antibiotics in the fleshy scales may have an influence on the relative resistance of the tissue to the three pathogens.

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